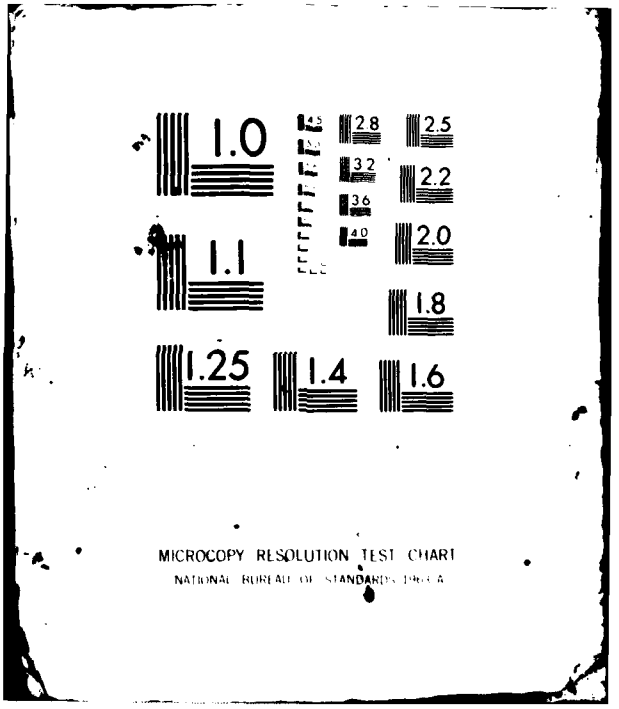
AD-A109 773 DEFENCE RESEARCH ESTABLISHMENT SUFFIELD RALSTON (ALBERTA) F/G 6/5
THE ADHERENCE OF NESSERIA MENINGITIDIS AND NEISSERIA GONORRHOEA--ETC(U)
AUG 81 L A WHITE, B E HOLBEIN
UNCLASSIFIED DRES-307 NL

END
DATE FILMED
2 82
DTIC


1.0
1.1
1.25
1.4
1.6
2.8
3.2
3.6
4.0
2.5
2.2
2.0
1.8
MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1963-A

UNCLASSIFIED

DRES

SUFFIELD REPORT

NO. 307

AD A109773

(3) LEVEL II

THE ADHERENCE OF *NEISSERIA MENINGITIDIS* and *NEISSERIA GONORRHOEAE* TO HUMAN EMBRYONIC KIDNEY CELLS (U)

by

L.A. White and B.E. Holbein

DTIC ELECTE JAN 19 1982 S D B

Project No. 16A10

August 1981

DISTRIBUTION STATEMENT A
Approved for public release;
Distribution Unlimited

DTIC FILE COPY

DEFENCE RESEARCH ESTABLISHMENT SUFFIELD : RALSTON : ALBERTA

01 18 82 019

DEFENCE RESEARCH ESTABLISHMENT SUFFIELD
RALSTON ALBERTA

SUFFIELD REPORT NO. 307

THE ADHERENCE OF *NEISSERIA MENINGITIDIS* and *NEISSERIA GONORRHOEAE* TO HUMAN EMBRYONIC KIDNEY CELLS (U)

by

L.A. White and B.E. Holbein*

Project No. 16A10

* Present Address: Dept. of Microbiology and Immunology, McGill University, Montreal, P.Q.

DEFENCE RESEARCH ESTABLISHMENT SUFFIELD
RALSTON ALBERTA

SUFFIELD REPORT NO. 307

THE ADHERENCE OF *NEISSERIA MENINGITIDIS* and *NEISSERIA GONORRHOEAE* TO HUMAN EMBRYONIC KIDNEY CELLS (U)

by

L.A. White and B.E. Holbein

ABSTRACT

The *in vitro* adherence of *N. meningitidis* isolates and *N. gonorrhoeae* colonial types to human embryonic kidney (HEK) cells was examined. Two of five serogroup B nasopharyngeal *N. meningitidis* isolates of different serotype adhered to HEK cells, after one subculture from their initial isolation. All of the isolates possessed substantial numbers of pili after one subculture, and these results suggest that pili are not the sole mediators of adherence of *N. meningitidis* to HEK cells. Both pili and the ability to adhere were lost during seven subculturings. *N. gonorrhoeae* colonial type T_1 cells were found to readily adhere to HEK cells and to be heavily piliated. Non piliated *N. gonorrhoeae* colonial type T_4 cells were incapable of adherence to HEK cells.

(U)

ACKNOWLEDGEMENTS

We acknowledge the capable technical assistance of Miss L. Mofford and Mr. G. Park. Thanks are expressed to Dr. E. Wagenaar, University of Lethbridge, for the use of electron microscopic facilities. Ms. E.B. Mackie, University of Calgary, is thanked for the serotyping of the serogroup B, *N. meningitidis* isolates employed in this study.

DEFENCE RESEARCH ESTABLISHMENT SUFFIELD
RALSTON ALBERTA

SUFFIELD REPORT NO. 307

THE ADHERENCE OF *NEISSERIA MENINGITIDIS* and *NEISSERIA GONORRHOEAE* TO HUMAN EMBRYONIC KIDNEY CELLS (U)

by

L.A. White and B.E. Holbein

INTRODUCTION

Most bacterial infections of man and animals have their beginnings on the mucosal surfaces of the respiratory, gastrointestinal, and urogenital tracts. It is on these surfaces that a bacterial pathogen has to proliferate, and/or overcome host defence mechanisms, compete with host commensal microorganisms, and penetrate into the body before disease becomes evident. Adherence of bacteria to mucosal surfaces may be of fundamental importance in the disease process and adherence of a variety of bacteria has received considerable attention in recent years (1 - 8).

By far the most intensive research on bacterial adherence has concerned *Neisseria gonorrhoeae*. Kellogg *et al.* (9) demonstrated that four main colonial types can segregate after initial isolation, and that these colonial types differed in virulence. Colonial types 1 and 2 have been shown to differ from types 3 and 4, in that they possess pili (10), are more virulent (9), and adhere more readily to a variety of animal cells (11, 12, 13, 14).

These studies have provided indirect evidence that pili mediate adherence to tissues, and thus constitute a virulence determinant of *N. gonorrhoeae*. The ability of isolated gonococcal pili to agglutinate human erythrocytes (15), and the concomitant transformation of piliation and virulence into *N. gonorrhoeae* type 4 (16) further strengthens this hypothesis. The studies of Baron and Saz (16) indicate that at the very least pili production is a marker for a virulence determinant. However, antigen(s) distinct from pili, and involved with gonococcal cell interactions, must exist, since pili were found to play only a minor role in the association of gonococci with polymorphonuclear neutrophils (5, 17), and non-piliated forms of gonococci have been shown to adhere to other cell types (11, 13).

In our own laboratory, we are interested in the roles that surface structures (e.g., pili) might play in the virulence of *N. meningitidis*. To this end, we are investigating models for the study of virulence factors. This report describes results obtained using a human embryonic kidney (HEK) cell adherence model. The adherence of *N. gonorrhoeae* colony types was also examined for purposes of comparison (a preliminary report of this study was presented at the 20th annual meeting, Canadian Federation of Biological Societies, 1977).

MATERIALS AND METHODS

Preparation of Bacteria for Adherence Studies

N. gonorrhoeae strain 74002 (obtained from the Laboratory Centre for Disease Control, National Health and Welfare, Ottawa, Canada) was grown on GC agar (Difco, Detroit, Michigan), supplemented with Isovitalex (Baltimore Biological Laboratory (BBL), Cockeysville, Md.), at 35°C, in an atmosphere of 5% CO_2 in air. Colonial types T_1 and T_4 were allowed to segregate, and were selectively transferred until colonial type stability had been achieved (18). The colony types were lyophilized. Growth from the lyophilized cultures displayed approximately 95% colonial type purity after 18 h growth. Loopsfull of the appropriate colony types were suspended in neisseria chemically defined medium (NCDM) (Grand Island Biological Company, Grand Island, N.Y. (GIBCO)), and were resuspended with glass beads (5 mm diameter) for 15 sec. The resulting suspensions were passed through cotton filters to remove clumps of bacteria.

N. meningitidis isolates were obtained by means of cotton swabs from the throats of 5 healthy male recruits at Canadian Forces Base St. Jean. All isolates were found to be serogroup B *N. meningitidis*, and have been designated SJ-1749, SJ-1766, SJ-1770, SJ-1776, and SJ-1779. The swabs were immediately placed in tubes containing 3 mL trypticase soy broth (BBL) and 10% dimethyl sulphoxide (Fisher Scientific Co. Ltd.,

Canada), frozen in dry ice and held at −60°C until required. The tubes were rapidly thawed at 45°C and the swabs were streaked on Columbia blood agar (CBA) (Columbia blood agar base (GIBCO), with 4% defribrinated sheep's red blood cells, and Isovitalex), containing VCNT antibiotic mixture (GIBCO; vancomycin 3 μg per mL, colistimethate 7.5 μg per mL, nystatin 12.5 U per mL, trimethoprim 5 μg per mL). Cultures were incubated as for *N. gonorrhoeae*. After 18 h incubation, single colonies were transferred to fresh plates and grown for 18 h, checked for purity, and prepared for adherence studies in the same manner as for *N. gonorrhoeae*. The cultures were also transferred daily for 7 days and re-examined for their ability to adhere to HEK cells.

Cell counts in bacterial suspensions were determined as colony forming units after plating on CBA.

The serogroup B, *N. meningitidis* strains isolated from recruits were serotyped in accordance with the system of Frasch and Chapman (19, 20, 21) by the ELISA method as described earlier (22). Antisera were raised in male Belted Dutch rabbits. Serotypes were as follows: strains SJ-1749 and -1770 (serotype 7), strain -1776 (serotype 8, 6, 7), strain -1779 (serotype 11, 7) and strain -1766 (serotype 6, 7).

Preparation of HEK Cell Cultures for Adherence Studies

Primary cell suspensions of HEK were obtained from GIBCO. Monolayers were grown in glass roller bottles (1500 cm^2, New Brunswick Scientific Co., New Brunswick, N.J.), at 35°C in an atmosphere of 5% CO_2 in air, using supplemented Hank's minimal essential medium (MEM) (Flow Laboratories, Rockville, Md., containing 10% fetal calf serum (FCS) (GIBCO) and 100 U per mL penicillin-streptomycin (BBL)). The monolayers were trypsinized (0.05% trypsin (BBL) at pH 8.0 and 35°C), the cells were washed once in MEM, and the washed cells were resuspended at approximately 10^6 cells per mL in MEM plus 10% DMSO. The cell suspensions were then frozen at −20°C for 1 h, followed by −70°C for 4 h, and held at −100°C until required.

Frozen cells were thawed rapidly at 45°C, and were washed and resuspended at approximately 10^6 cells per mL in RPMI (Flow; RPMI 1640 medium, with glutamine, hepes buffer, 10% FCS, and 100 U per mL penicillin-streptomycin). Tissue culture chamber-slides (Lab-Tek® , 8 chamber, Miles Laboratories, Westmont, Ill.), containing 0.5 mL RPMI per chamber, were inoculated with 0.05 mL of HEK cell suspension. The slide cultures were incubated at 35°C in an atmosphere of 5% CO_2 in air for 24 h. The medium was then replaced with fresh RPMI and incubation was continued for a further 24 h. This technique produced well separated cells adhering to the glass slide at the bottom of the chambers. One hour prior to use, the medium was replaced with antibiotic-free RPMI.

Evaluation of *N. meningitidis* and *N. gonorrhoeae* Adherence to HEK Cells

HEK cell cultures were washed *in situ* with 0.5 mL NCDM, and overlaid with 0.2 mL of a bacterial suspension (approximately 10^7 cells per mL in NCDM). At 0.5 h intervals, over 3 h, the bacterial suspensions were removed and the HEK cell cultures were washed twice with 0.5 mL NCDM, and stained with Giemsa's stain for 30 min. The cultures were then examined and photographed using a Zeiss Photomicroscope III (Carl Zeiss Inc., Canada). Experiments reported here were repeated at least 2 times for each bacterial isolate.

Electron Microscopy

Samples of bacterial suspensions were taken onto carbon-coated, parlodion-backed grids, stained with 0.1% phosphotungstic acid, and examined in an AEI EM6-B electron microscope.

RESULTS

Adherence of *N. meningitidis* Isolates to HEK Cells

High numbers of cells of two of the five *N. meningitidis* isolates (SJ-1749 and SJ-1779) were found to adhere to HEK cells. Adherence was readily observed within 30 min of incubation and increased during the 3 h incubation period. Greater than 90% of the HEK cells had high numbers of meningococci (>100 diplococci) adhering to them by 2 h incubation. Figures 1a and 1c are representative examples of the degree of adherence observed. In our method, adherence of meningococci to the glass slides was extremely low and consequently such background did not interfere with interpretation of results. Adherence was not detected at appreciable numbers for the other three isolates during the 3 h incubation period. The results obtained with SJ-1766 and SJ-1770 are shown in Figures 2a and 2c. Similar results (not shown) were obtained with SJ-1776. All five isolates were found to be piliated and to possess abundant extracellular material, and what appeared to be cell wall blebs (Figs. 1b, 1d, 2b, and 2d).

Pili were most often seen in large bundles (Figs. 1b and 2d) which were usually poorly resolved. However, individual component pili could be observed where the bundles had frayed (Fig. 2d). The frayed bundle shown in Figure 2d revealed that the bundle was composed of a great many individual pili. The abundant extracellular material observed on the isolates can be seen in Figure 1d. In this case, the preponderance of cell wall blebs (B), extracellular material (C), and bundles of pili precluded the resolution of the cell outline. (Note that the magnification for Figure 1d is identical to those for Figures 1b, 2b, and 2d.)

It is interesting to note that isolates SJ-1749 and -1770 which were of the same serotype, 7, and which possessed pili in large bundles (Figs. 1b and 2d, respectively) did not show similar degrees of adherence. The former isolate adhered strongly whereas adherence of SJ-1770 was minimal.

None of the five isolates of *N. meningitidis* adhered to HEK cells after a total of 7 subcultures (results not shown). In addition there was a total disappearance of pili during subculture (Fig. 3). Extracellular material and cell wall blebs were also less evident.

Adherence of *N. gonorrhoeae* Colonial Types to HEK Cells

Colonial type T_1 *N. gonorrhoeae* cells were found to adhere readily to HEK cells, and to be heavily piliated (Figs. 4a and 4b). Adherence was as great as that observed with *N. meningitidis* isolates SJ-1749 and SJ-1779, and similar levels of adherence were observed in over 90% of the HEK cells. Colonial type T_4 *N. gonorrhoeae* cells did not adhere (Fig. 4c), nor did they possess pili (Fig. 4d).

DISCUSSION

Model systems of infection, in the case of *N. meningitidis* and *N. gonorrhoeae*, may represent the only fruitful approach for elucidating the virulence determinants of these organisms. In the present study, adherence of *N. meningitidis* to HEK cells was studied with the express purpose of examining what role, if any, pili might play in adherence to epithelial tissue.

HEK cells were selected for this study for several reasons:

1) being human in origin, they represented a cell type of the natural host;

2) they are epithelial cells, and *N. meningitidis* infection is presumed to originate on nasal mucosal epithelial tissue;

3) being embryonic, HEK cells should be devoid of any immune mechanisms available to the natural host;

4) well separated cells were readily obtained in tissue culture using the simple procedures outlined; and

5) stock cultures of HEK cells were easily preserved by the freezing method outlined.

Well separated HEK cells, as opposed to complete monolayers, were used in the adherence studies to allow for an accurate assessment of adherence to individual cells and to avoid possible confusion due to encroaching cells.

Two of five serogroup B nasopharyngeal isolates, taken from the same class of recruits, were found to readily adhere to HEK cells, after one subculture from initial isolation. These results indicated that the adhering isolates possessed characteristics which were not possessed by those isolates incapable of adherence, and that presumbly these characteristics resided on the bacterial surface. However, all the isolates appeared to be ultrastructurally similar, and all possessed substantial numbers of pili, cell wall blebs (similar to those observed by DeVoe and Gilchrist (23)), and considerable quantities of extracellular material. These results strongly suggest that pili are not mediators for adherence of *N. meningitidis* to HEK cells. A special pilar type, involved in adherence, would have to be postulated in order to maintain that pili were mediators for adherence in this case.

It was also obvious that a similar antigenic structure, according to the system of Frasch and Chapman (19, 20, 21), did not result in a similar degree of adherence. No isolates possessing the serotype 2 antigen, which has been claimed to be a virulence marker, were included in this study, however, and further study of fresh isolates (i.e. less than 2 subcultures removed from initial isolation) of this serotype may reveal a positive correlation with adherence.

The large bundles of pili observed on these isolates have not been observed during a previous study of many primary cultures of *N. meningitidis* (23), although they have been previously observed in association with *N. gonorrhoeae* (24). The ability to adhere was lost from the *N. meningitidis* isolates during seven subcultures. There was also a concomitant loss of pili from all the isolates, a phenomenon which had previously been reported to occur in as few as two subcultures (23).

Our results with *N. gonorrhoeae* are in general agreement with the results of previous studies (11, 12, 13, 14), and support the contention that gonococcal pili mediate adherence to cells.

The present is the first report of adherence of *N. meningitidis* to animal cells *in vitro*. Although absolute differences in the ability to adhere to HEK cells were observed in a set of serogroup B isolates, indicating that variations within a serogroup must exist, any relationship between adherence and virulence of *N. meningitidis* remains to be established. Studies using suitable infection models will hopefully be useful in establishing any relationship between adherence of *N. meningitidis* to epithelial cells *in vitro* and virulence *in vivo*, and we are presently working in this direction.

REFERENCES

1. Aly, R., H.I. Shinefield, W.G. Strauss and H.I. Maibach. 1977. Bacterial adherence to nasal mucosal cells. Infect. Immun. **17**: 546 - 549.

2. Freter, R. and G.W. Jones. 1976. Adhesive properties of *Vibrio cholerae*: Nature of the interaction with intact mucosal surfaces. Infect. Immun. **14**: 246 - 256.

3. Frost, A.J. 1975. Selective adhesion of microorganisms to the ductular epithelium of the bovine mammary gland. Infec. Immun. **12**: 1154 - 1156.

4. Hohmann, A. and M.R. Wilson. 1975. Adherence of enteropathogenic *Escherichia coli* to intestinal epithelium *in vivo*. Infect. Immun. **12**: 866 -880.

5. King, G., J.F. James and J. Swanson. 1978. Studies on gonococcus infection XI. Comparison of *in vivo* and *in vitro* association of *Neisseria gonorrhoeae* with human neutrophils. J. Infect. Dis. **137**: 38 - 43.

6. Powell, D.A., P.C. Hu, M. Wilson, A.M. Collier and J.B. Baseman. 1976. Attachment of *Mycoplamsa pneumoniae* to respiratory epithelium. Infect. Immun. **13**: 959 - 966.

7. Smith, H. 1977. Microbial surfaces in relation to pathogenicity. Bacteriol. Rev. **41**: 475 - 500.

8. Tebutt, G.M., D.R. Veale, J.G.P. Hutchison and H. Smith. 1976. The adherence of pilate and non-pilate strains of *Neisseria gonorrhoeae* to human and guinea-pig epithelial tissues. J. Med. Microbiol. **9**: 263 - 273.

9. Kellogg, D.S., I.R. Cohen, L.C. Norins, A.L. Schroeter and G. Reising. 1968. *Neisseria gonorrhoeae II*. Colonial variation and pathogenicity during 35 months *in vitro*. J. Bacteriol. **96**: 596 - 605.

10. Swanson, J., M.D. Stephen, J. Kraus and E.C. Gotschlich. 1971. Studies on gonococcus infection I. Pili and zones of adhesion: Their relationship to gonococcal growth patterns. J. Exp. Med. **134**: 886 - 906.

11. Brodeur, B.R., W.M. Johnson, K.G. Johnson and B.B. Diena. 1977. *In vitro* interaction of *Neisseria gonorrhoeae* type 1 and type 4 with tissue culture cells. Infect. Immun. **15**: 560 - 567.

REFERENCES (Cont'd)

12. James-Holmquest, A.N., J. Swanson, T.M. Buchanan, R.D. Wende and R.P. Williams. 1974. Differential attachment by piliated and nonpiliated *Neisseria gonorrhoeae* to human sperm. Infect. Immun. **9**: 897 - 902.

13. James, A.N., J.M. Knox and R.P. Williams. 1976. Attachment of gonoccocci to sperm. Influence of physical and chemical factors. Brit. J. Ven. Dis. **52**: 128 -135.

14. Swanson, J. 1973. Studies on gonococcus infection IV. Pili: their role in attachment of gonococci to tissue culture cells. J. Exp. Med. **137**: 571 - 589.

15. Buchanan, T.M. and W.A. Pearce. 1976. Pili as a mediator of the attachment of gonococci to human erythrocytes. Infect. Immun. **13**: 1483 - 1489.

16. Baron, E.S. and A.K. Saz. 1978. Genetic transformation of piliation and virulence into *Neisseria gonorrhoeae* T_4. J. Bacteriol. **133**: 972 - 986.

17. Swanson, J., E. Sparks, D. Young and G. King. 1975. Studies on gonococcus infection X. Pili and leukocyte association factor as mediators of interactions between gonococci and eukaryotic cells *in vitro*. Infect. Immun. **11**: 1352 - 1361.

18. Novotny, P., J.A. Short, M. Hughes, J.J. Miler, C. Syrett, W.H. Turner J.R.W. Harris and I.P.B. MacLennan. 1977. Studies on the mechanism of pathogenicity of *Neisseria gonorrhoeae*. J. Med. Microbiol. **10**: 347 - 365.

19. Frasch, C.E. and S.S. Chapman. 1972. Classification of *Neisseria meningitidis* group B into distinct serotypes. I. Serological typing by a microbactericidal method. Infect. Immun. **5**: 98 - 102.

20. Frasch, C.E. and S.S. Chapman. 1972. Classification of *Neisseria meningitidis* group B into distinct serotypes. II. Extraction of type-specific antigens for serotyping by precipitin techniques. Infect. Immun **6**: 127 - 133.

21. Frasch, C.E. and S.S. Chapman. 1973. Classification of *Neisseria meningitidis* group B into distinct serotypes. III. Application of new bactericidal-inhibition technique to distribution of serotypes among cases and carriers. J. Infect. Dis. **127**: 149 - 154.

REFERENCES (Cont'd)

22. Mackie, E.B., L.A. White, K.N. Brown and L.E. Bryan. 1981. Enzyme-linked immunosorbent assay compared with immunoprecipitation for serotyping *Neisseria meningitidis* and its use in demonstrating serotype polymorphism. J. Clin. Microbiol. **13**: 449 - 455.

23. DeVoe, I.W. and J.E. Gilchrist. 1975. Pili on meningococci from primary cultures of nasopharyngeal carriers and cerebrospinal fluid of patients with acute disease. J. Exp. Med. **141**: 297 - 305.

24. Jephcott, A.E., A. Reyn and A. Birch-Anderson. 1971. *Neisseria gonorrhoeae III.* Demonstration of presumed appendages to cells from different colony types. Acta Pathol. Microbiol. Scand. Sect. B. **79**: 437 - 439.

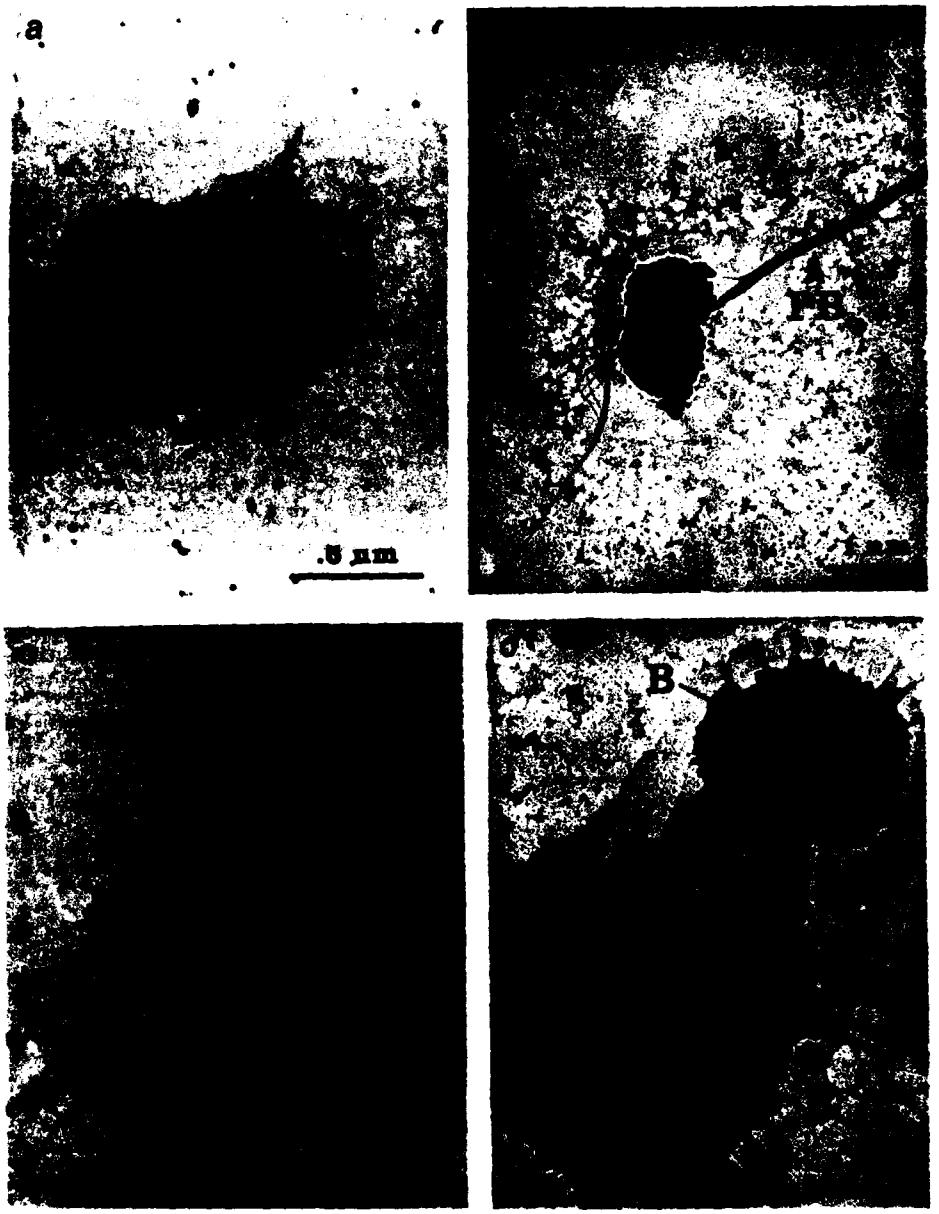


Figure 1

Adherence of *N. meningitidis* isolates SJ-1749 and SJ-1779 to HEK cells. *N. meningitidis* isolates were subcultured once after initial isolation, and allowed to adhere to HEK cells for 2 h. Figures 1a and 1c: light micrographs of SJ-1749 and SJ-1779 respectively (both 5,000×). Figures 1b and 1d: electron micrographs of SJ-1749 and SJ-1779 respectively (both 15,000×).

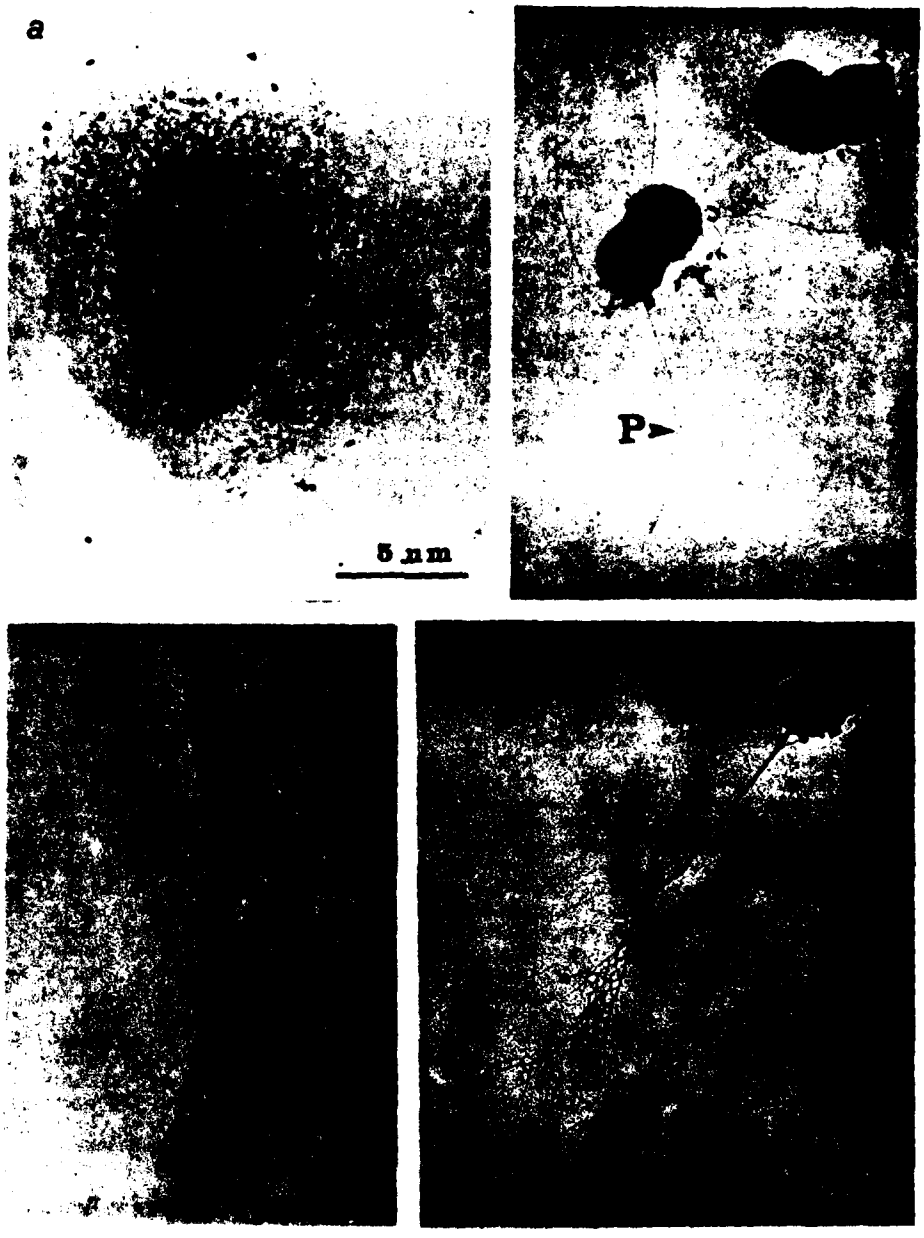


Figure 2

Adherence of *N. meningitidis* isolates SJ-1766 and SJ-1770 to HEK cells. *N. meningitidis* isolates were subcultured once after initial isolation and allowed to adhere to HEK cells for 2 h. Figures 2a and 2c: light micrographs of SJ-1766 and SJ-1770 respectively (both 5,000 ×). Figures 2b and 2d: electron micrographs of SJ-1766 and SJ-1770 respectively (both 15,000 ×).

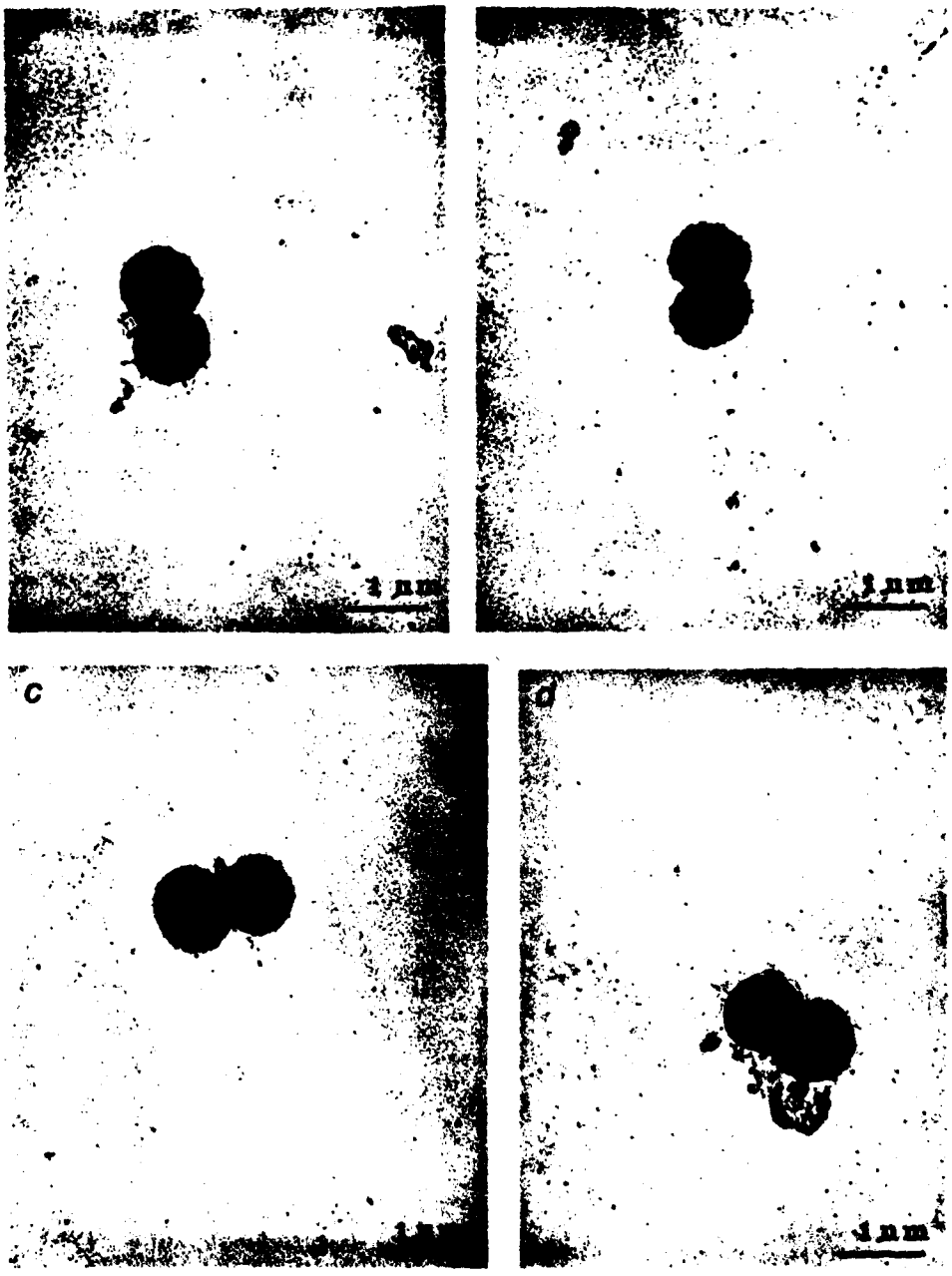


Figure 3

Appearance of *N. meningitidis* isolates after seven subcultures. Figure 3a: SJ-1749; 3b: SJ-1779; 3c: SJ-1766: and 3d: SJ-1770 (all 15,000×).

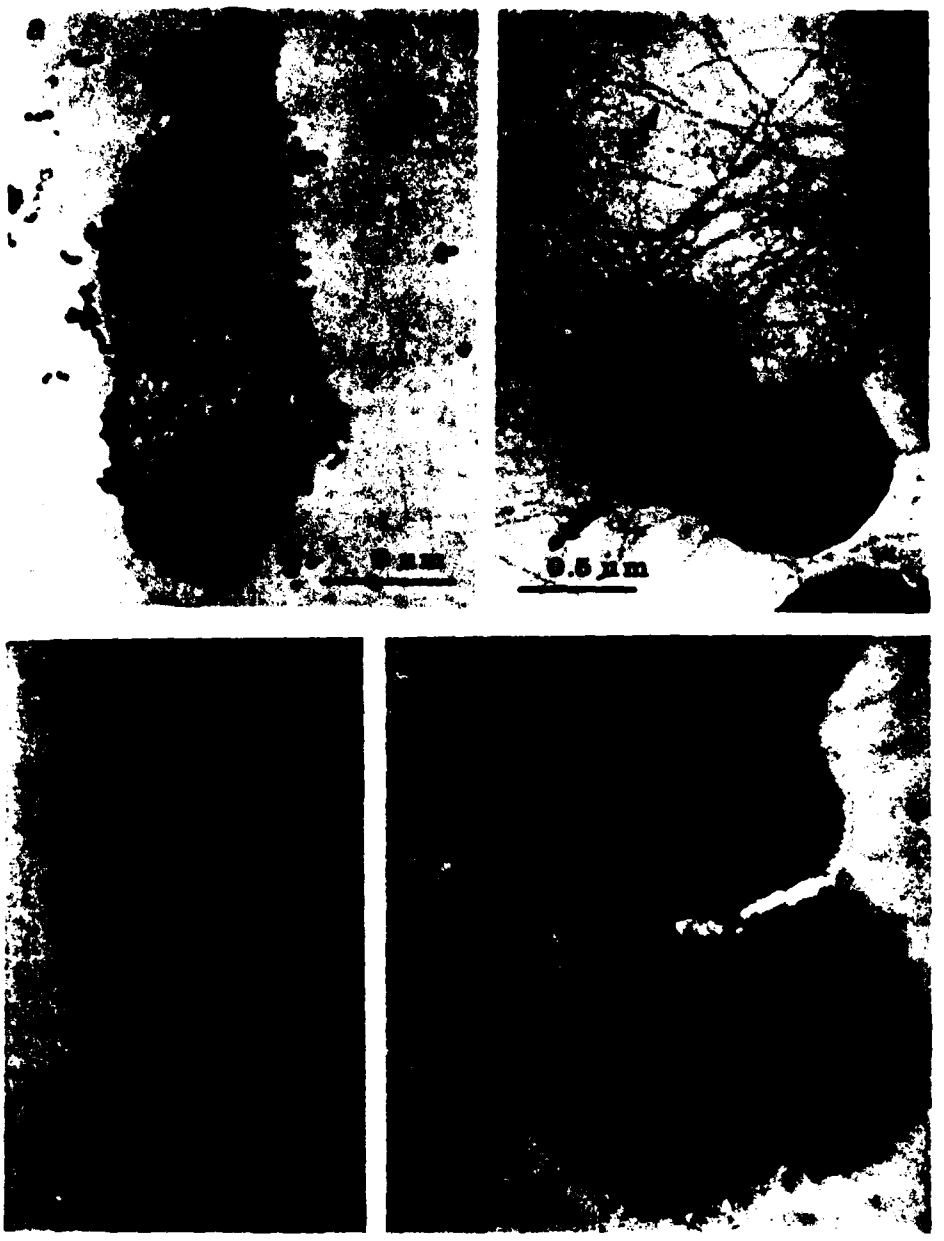


Figure 4

Adherence of *N. gonorrhoeae* colonial types T_1 and T_4 to HEK cells. Cells of *N. gonorrhoeae* colonial types T_1 and T_4 were allowed to adhere to HEK cells for 2 h. Figures 4a and 4c: light micrographs of T_1 and T_4 respectively (both 5,000 ×). Figures 4b and 4d: electron micrographs of T_1 and T_4 cells respectively (both 45,000 ×).

UNCLASSIFIED
This Sheet Security Classification

DOCUMENT CONTROL DATA - R & D
(Security classification of title, body of abstract and indexing annotation must be entered when the overall document is classified)

1 ORIGINATING ACTIVITY
DEFENCE RESEARCH ESTABLISHMENT SUFFIELD

2a. DOCUMENT SECURITY CLASSIFICATION
UNCLASSIFIED

2b. GROUP

3 DOCUMENT TITLE
THE ADHERENCE OF NEISSERIA MENINGITIDIS AND NEISSERIA GONORRHOEAE TO HUMAN EMBRYONIC KIDNEY CELLS (U)

4 DESCRIPTIVE NOTES (Type of report and inclusive dates)
SUFFIELD REPORT

5 AUTHOR(S) (Last name, first name, middle initial)
L.A. White and R.E. Holbein

6 DOCUMENT DATE
August 1981

7a. TOTAL NO. OF PAGES
12

7b. NO. OF REFS
24

8a. PROJECT OR GRANT NO.
16A10

9a. ORIGINATOR'S DOCUMENT NUMBER(S)
SUFFIELD REPORT NO. 307

8b CONTRACT NO.

9b. OTHER DOCUMENT NO.(S) (Any other numbers that may be assigned this document)

10. DISTRIBUTION STATEMENT
UNLIMITED DISTRIBUTION

11. SUPPLEMENTARY NOTES

12. SPONSORING ACTIVITY

13. ABSTRACT

The in vitro adherence of N. meningitidis isolates and N. gonorrhoeae colonial types to human embryonic kidney (HEK) cells was examined. Two of five serogroup B nasopharyngeal N. meningitidis isolates of different serotypes adhered to HEK cells, after one subculture from their initial isolation. All of the isolates possessed substantial numbers of pili after one subculture, and these results suggest that pili are not the sole mediators of adherence of N. meningitidis to HEK cells. Both pili and the ability of adhere were lost during seven subculturings. N. gonorrhoeae colonial type T_1 cells were found to readily adhere to HEK cells and to be heavily piliated. Non piliated N. gonorrhoeae colonial type T_4 cells were incapable of adherence to HEK cells.

FORM B-4

DSIS
79-010

KEY WORDS

Neisseria
meningitis
gonorrhea
virulence
pili
adherence

INSTRUCTIONS

1. ORIGINATING ACTIVITY: Enter the name and address of the organization issuing the document.

2a. DOCUMENT SECURITY CLASSIFICATION: Enter the overall security classification of the document including special warning terms whenever applicable.

2b. GROUP: Enter security reclassification group number. The three groups are defined in Appendix 'M' of the DRB Security Regulations.

3. DOCUMENT TITLE: Enter the complete document title in all capital letters. Titles in all cases should be unclassified. If a sufficiently descriptive title cannot be selected without classification, show title classification with the usual one-capital-letter abbreviation in parentheses immediately following the title.

4. DESCRIPTIVE NOTES: Enter the category of document, e.g. technical report, technical note or technical letter. If appropriate, enter the type of document, e.g. interim, progress, summary, annual or final. Give the inclusive dates when a specific reporting period is covered.

5. AUTHOR(S): Enter the name(s) of author(s) as shown on or in the document. Enter last name, first name, middle initial. If military, show rank. The name of the principal author is an absolute minimum requirement.

6. DOCUMENT DATE: Enter the date (month, year) of Establishment approval for publication of the document.

7a. TOTAL NUMBER OF PAGES: The total page count should follow normal pagination procedures, i.e., enter the number of pages containing information.

7b. NUMBER OF REFERENCES: Enter the total number of references cited in the document.

8a. PROJECT OR GRANT NUMBER: If appropriate, enter the applicable research and development project or grant number under which the document was written.

8b. CONTRACT NUMBER: If appropriate, enter the applicable number under which the document was written.

9a. ORIGINATOR'S DOCUMENT NUMBER(S): Enter the official document number by which the document will be identified and controlled by the originating activity. This number must be unique to this document.

9b. OTHER DOCUMENT NUMBER(S): If the document has been assigned any other document numbers (either by the originator or by the sponsor), also enter this number(s).

10. DISTRIBUTION STATEMENT: Enter any limitations on further dissemination of the document, other than those imposed by security classification, using standard statements such as:

(1) "Qualified requesters may obtain copies of this document from their defence documentation center."

(2) "Announcement and dissemination of this document is not authorized without prior approval from originating activity."

11. SUPPLEMENTARY NOTES: Use for additional explanatory notes.

12. SPONSORING ACTIVITY: Enter the name of the departmental project office or laboratory sponsoring the research and development. Include address.

13. ABSTRACT: Enter an abstract giving a brief and factual summary of the document, even though it may also appear elsewhere in the body of the document itself. It is highly desirable that the abstract of classified documents be unclassified. Each paragraph of the abstract shall end with an indication of the security classification of the information in the paragraph (unless the document itself is unclassified) represented as (TS), (S), (C), (R), or (U).

The length of the abstract should be limited to 20 single-spaced standard typewritten lines, 7½ inches long.

14. KEY WORDS: Key words are technically meaningful terms or short phrases that characterize a document and could be helpful in cataloging the document. Key words should be selected so that no security classification is required. Identifiers, such as equipment model designation, trade name, military project code name, geographic location, may be used as key words but will be followed by an indication of technical context.